PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference MON/P102611WO	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/GB2004/002364	International filing date *(day/month/year)* 04.06.2004	Priority date *(day/month/year)* 12.06.2003
International Patent Classification (IPC) or national classification and IPC G01N33/545, B05D7/24, C12N5/00		
Applicant PLASSO TECHNOLOGY LTD		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 5 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20.07.2005	10.11.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Luis Alves, D Telephone No. +49 89 2399-8953

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/002364

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-23	as originally filed
Claims, Numbers	
1, 3-44	received on 25.07.2005 with letter of 20.07.2005
Drawings, Figures	
1-3	as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 2
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/002364

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-15, 17-19, 21-32, 43, 44
	No:	Claims	1, 16, 10, 33-42
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1, 3-44
Industrial applicability (IA)	Yes:	Claims	1, 3-44
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

Reference is made to the following documents cited in the International search report:

D1: GOESSL ANDREAS ET AL: "Plasma lithography: Thin-film patterning of polymers by RF plasma polymerization II: Study of differential binding using adsorption probes" JOURNAL OF BIOMATERIALS SCIENCE POLYMER EDITION, vol. 12, no. 7, 2001, pages 739-753, XP009045548 ISSN: 0920-5063

D3: WHITTLE J D ET AL: "A method for the deposition of controllable chemical gradients" CHEMICAL COMMUNICATIONS 21 JUL 2003 UNITED KINGDOM, vol. 9, no. 14, 21 July 2003 (2003-07-21), pages 1766-1767, XP008045537 ISSN: 1359-7345

D4: WO 03/082483 A (PLASSO TECHNOLOGY LIMITED; SHORT, ROB; WHITTLE, JASON; SHARD, ALEX, G;) 9 October 2003 (2003-10-09)

Section V:

1. Present claim 1 encompasses two embodiments: The substrate obtainable by the process involving steps (i) and (iii) on the other hand, and the substrate obtainable by the process involving steps (ii) and (iii) on the hand.

   D1 discloses the preparation of a surface displaying a pattern of areas with affinity for cells (see abstract). The surface was prepared by taking a surface having a pattern of fluorocarbon plasma polymer and applying thereto a surfactant conjugated peptide. The peptide is thus immobilised on the surface, in a pattern, via the surfactant which has a strong affinity for the fluorocarbon polymer (see abstract).
   Therefore, D1 discloses a surface of a substrate having a plasma polymer deposited thereon in a non-uniform way and on that non-uniform surface a binding entity (the peptide).

A method using two monomers, their relative proportion being varied along the surface of the substrate, resulting in a plasma polymerised surface displaying a gradient of functional groups along its surface, followed by the introduction of a biomolecule, resulting in a surface displaying a gradient of said biomolecule along the surface, is not disclosed in D1.

However, the method and the surface disclosed in D1 fall within the scope of independent claims 1, 36, 37, 40 and 42 in the present application. Said claims do not comply with the requirements of Article 33(2) PCT.
The features in dependent claims 16, 20, 33 to 35 and 38, 39 and 41 are already disclosed in D1. Thus, said claims do not comply with the requirements of Article 33(2) PCT.
Dependent claims 3 to 15, 17 to 19 and 21 to 32 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step (Article 33(3) PCT).
Independent claims 43 and 44 concern uses of the surfaces already known from D1, as discussed above. D1 is concerned with the preparation of surfaces for cell culture. Therefore, the uses defined in claims 43 and 44 are obvious uses of such surfaces and therefore said claims do not involve an inventive step (Article 33(3) PCT).

2. Document D3, cited in the International search report as an intermediate document, was published on the web on 18 June 2003. D3 is not to be taken into account for the assessment of novelty and presence of an inventive step because the priority date of 12 June 2003 is validly claimed by the present application.

Section VI:

1. Document D4 is a patent document cited in the International search report as an intermediate document. D4 was published on 9 October 2003, filed on 24 March 2003 and claims a priority date of 28 March 2002.
At the national /regional phase this document may become relevant to the question of

novelty of claims the present claims.

CLAIMS

1. A substrate obtainable by

i.) depositing on a surface of a substrate at least one plasma monomer from a monomer source wherein during deposition of said monomer said monomer and/or said surface are moved relative to one another to provide a non-uniform plasma polymerised surface; or

ii) depositing on the surface at least one plasma monomer from at least two spatially separated monomer sources to provide a non-uniform plasma polymerised surface; and

iii) introducing to at least part of said plasma polymerised surface a binding entity to provide a non-uniform surface formed from said binding entity

wherein the binding entity provides a surface onto which a cell can grow or attach.

3. A substrate as claimed in claim 1 wherein the binding entity comprises a carboxyl or amine functional group.

4. A substrate as claimed in claim 1 wherein the binding entity is selected from the group consisting of cells, metabolites, pharmaceutically active agents, proteins including hormones, antibodies, enzyme, receptor; macromolecules including DNA, RNA, protein fragments, peptides, polypeptides; ligands, proteoglycans, carbohydrates, nucleotides, oligonucleotides, toxic reagents and chemical species.

5. A substrate as claimed in claim 1 wherein the binding entity comprises an immobilised or adsorbed biological entity.

6. A substrate as claimed in claim 5 wherein the biological entity is a protein or protein fragment.

7. A cell substrate as claimed in claim 1 wherein the binding entity interacts covalently with functional groups of the plasma polymerised surface.

8. A substrate as claimed in claim 1 wherein the binding entity is immobilised on the plasma polymer surface.

9. A substrate as claimed in claim 1 wherein the binding entity is chemically linked to functional groups in the plasma polymer surface.

10. A substrate as claimed in claim 1 wherein the binding entity interacts non-covalently with functional groups of the plasma polymerised surface.

11. A substrate as claimed in claim 1 wherein a cell interacts with the binding entity of the plasma polymerised surface.

12. A substrate as claimed in claim 1 wherein the monomer is a volatile alcohol.

13. A substrate as claimed in claim 1 wherein the monomer is a volatile acid.

14. A substrate as claimed in claim 1 wherein the monomer is a volatile amine.

15. A substrate as claimed in claim 1 wherein the monomer is a volatile hydrocarbon.

16. A substrate as claimed in claim 1 wherein the monomer is a volatile fluorocarbon.

17. A substrate as claimed in claim 1 wherein the monomer is an ethyleneoxide-type molecule.

18. A substrate as claimed in claim 1 wherein the monomer is a volatile siloxane.

19. A substrate as claimed in claim 1 wherein the monomer is selected from the group consisting of N-vinyl pyrolidone, allyl alcohol; acrylic acid; octa-1,7-diene; allyl amine; perfluorohexane; tetraethyleneglycol monoallyl ether and hexamethyl disiloxane (HMDSO).

20. A substrate as claimed in claim 1 wherein the polymer consists of a single monomer.

21. A substrate as claimed in claim 18 wherein the monomer consists essentially of an ethylenically unsaturated organic compound.

22. A substrate as claimed in claim 19 wherein the monomer consists essentially of a single ethylenically unsaturated organic compound.

23. A substrate as claimed in claim 20 wherein the monomer consists of an ethylene oxide type molecule.

24. A substrate as claimed in claim 19 wherein the monomer consists of a mixture of two or more ethylenically unsaturated organic compounds.

25. A substrate as claimed in claim 19 wherein the compound is selected from the group consisting of an alkene containing up to 20 carbon atoms, a carboxylic acid, an alcohol and an amine.

26. A substrate as claimed in claim 1 wherein the polymer is a co-polymer.

27. A substrate as claimed in claim 24 wherein the co-polymer comprises at least one organic monomer with at least one hydrocarbon.

28. A substrate as claimed in claim 1 wherein the monomer is a polymerisable monomer having a vapour pressure of at least 6.6×10^{-2} mbar.

29. A substrate as claimed in claim 1 wherein the monomer (s) is/are deposited on said surface in spatially separated dots.

30. A substrate as claimed in claim 1 wherein the monomer (s) is/are deposited on said surface in tracks or lines.

31. A substrate as claimed in claim 27 or 28 wherein the chemical composition and/or functionality of the line, track or dot is non-uniform along its length.

32. A substrate as claimed in claim 1 wherein the chemical composition and/or functionality of the line, track or dot is non-uniform in its height.

33. A substrate as claimed in claim 1 wherein the surface comprises non-plasma deposited regions that are comprised of polymerised ethylene-oxide type monomer to provide a non-binding surface.

34. A substrate as claimed in any preceding claim wherein the substrate is selected from the group consisting of glass, plastics, nitrocellulose, Poly vinylidene fluoride (PVdF), polycarbonate, poly (methylmathacrylate), nylon, metal, ceramics, quartz, composite structures and silicon wafer.

35. A substrate as claimed in claim 34 wherein the plastic is selected from the group consisting of polyethylene terephthalate, high density polyethylene, low density polyethylene, polyvinyl chloride, polypropylene and polystyrene.

36. A cell culture system comprising a substrate that includes a surface obtainable by depositing on at least part of at least one surface of said substrate a non-uniform plasma polymer surface.

37. A cell culture system comprising a substrate as claimed in any preceding claim.

38. A cell culture system as claimed in claim 36 or 37 wherein the system is part of an assay product.

39. A cell culture system as claimed in claim 38 wherein said assay product is a microarray.

40. A cell culture system as claimed in claim 38 wherein said assay product is a microtitre plate.

41. A cell culture system as claimed in claim 38 wherein said assay product comprises a microfluidic device or a part.

42. Use of a substrate as claimed in any of claims 1 to 35 in cell culture.

43. Use of a substrate as claimed in any of claims 1 to 35 in the separation of cells.

44. A method of screening biological molecules comprising the steps of

i) preparing a substrate as claimed in claim 1;

ii.) screening the surface of said substrate to determine the binding property of a cell to said surface, wherein said binding property is identifiable by its binding position on said surface; and

iii.) identifying the cell with said binding property.